AUTHORITY: Secs. 201, 501, 502, 503, 505, 510, 701 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321, 351, 352, 353, 355, 360, 371); secs. 215, 351, 352, 353, 361 of the Public Health Service Act (42 U.S.C. 216, 262, 263, 263a, 264).

SOURCE: 38 FR 32064, Nov. 20, 1973, unless otherwise noted.

CROSS REFERENCES: For U.S. Customs Service regulations relating to viruses, serums, and toxins, see 19 CFR 12.21—12.23. For U.S. Postal Service regulations relating to the admissibility to the United States mails see parts 124 and 125 of the Domestic Mail Manual, that is incorporated by reference in 39 CFR part 111.

Subpart A—Pertussis Vaccine

§ 620.1 Pertussis Vaccine.

The proper name of this product shall be ''Pertussis Vaccine'', which shall be an aqueous preparation of killed whole *Bordetella pertussis* bacteria. The vaccine may be precipitated or adsorbed and may be combined with other antigens.

[56 FR 63410, Dec. 4, 1991]

§ 620.2 Production.

(a) *Propagation of bacteria.* Human blood shall not be used in culture medium for propagating bacteria either for seed or for vaccine. The culture medium for propagating bacteria for vaccine shall not contain ingredients known to be capable of producing allergenic effects in human subjects, except blood or blood products from lower animals other than the horse. When blood or a blood product is used, it shall be removed by washing the harvested bacteria. The bacterial concentrate shall be free of extraneous bacteria, fungi, and yeasts, as demonstrated by microscopic examination and cultural methods.

(b) *Bacterial content.* (1) The opacity of the bacterial concentrate shall be determined in terms of the U.S. Opacity Standard not later than 2 weeks after the harvest of the bacteria and before any treatment capable of altering the opacity of the bacterial concentrate.

(2) The total immunizing dose of a vaccine prepared with whole bacteria shall contain (i) in the case of nonadsorbed vaccine no more bacteria than the equivalent of 60 opacity units and (ii) in the case of adsorbed vaccine no more than the equivalent of 48 opacity units.

(c) *Detoxification.* After removing a sample for purity testing, the bacteria shall be killed and detoxified either (1) by heating, (2) by addition of a chemical agent and appropriate aging, or (3) by any combination of the stated procedures. The procedure used shall be one that has been shown to have no adverse effect on required safety, purity, and potency.

(d) *Preservative.* The vaccine shall contain a preservative.

§ 620.3 U.S. Standard preparations.

(a) The U.S. Standard Pertussis Vaccine shall be used for determining the potency of Pertussis Vaccine.

(b) The U.S. Opacity Standard shall be used in estimating the bacterial content of the vaccine and of the challenge culture.

§ 620.4 Potency test.

The number of protective units of the total human immunizing dose shall be estimated for each lot of vaccine from the results of simultaneous intracerebral mouse protection tests of the vaccine under test and the U.S. Standard Pertussis Vaccine. The potency test shall be performed as follows:

(a) *Mice.* Healthy mice shall be used, all from a single strain and of the same sex, or an equal number of each sex in each group, with individual weight varying no more than 4 grams in a single test. In no event shall any of the mice weigh less than 10 grams or more than 20 grams. A system of randomization shall be used to distribute the mice into the groups, with respect to shelf position and to determine the order of challenge. There shall be at least 3 groups consisting of no less than 16 mice each, for each vaccine. In addition, there shall be at least 4 groups consisting of no less than 10 mice each, for control purposes: one group for the challenge dose and 3 groups for titrating the virulence of the challenge dose.

(b) *Vaccination.* (1) Five-fold serial dilutions of the vaccine to be tested and of the standard vaccine shall be made in 0.85 percent sodium chloride solution. The dilutions of the vaccine under test shall have the same protective unitage, based on an estimate of 12 units per total human immunizing dose, as the unitage of the corresponding dilution of the standard vaccine. Each mouse in each group for vaccination shall be injected intraperitoneally with 0.5 ml. of the appropriate dilution.

(2) The interval between vaccination and challenge shall be 14 to 17 days. At least 87.5 percent of the mice in each group shall survive the period between vaccination and challenge and each mouse challenged shall appear healthy.

(c) *The challenge.* (1) The challenge culture of *Bordetella pertussis* for each test shall be taken from a batch of cultures which have been maintained by a method, such as freeze-drying, that retains constancy of virulence.

(2) The challenge and virulence titration doses shall be prepared as follows: The bacteria shall be harvested from a 20 to 24 hour culture grown on Bordet-Gengou medium seeded from a rapidly growing culture less than 48 hours old and uniformly suspended in a solution containing 1.0 percent casein peptone and about 0.6 percent sodium chloride at pH 7.1±0.1. The suspension, freed from agar particles and clumps of bacteria, and adjusted to an opacity of 10 units, shall be diluted in the solution used for suspending the bacteria, to provide in a volume of 0.03 ml. (i) a challenge dose of 0.0001 opacity units (1:3000) and (ii) virulence titration doses of $^1/_{50}$, $^1/_{250}$ and $^1/_{1250}$ respectively of the challenge dose.

(3) Each vaccinated mouse shall be injected intracerebrally with the challenge dose. The four groups of control mice shall be injected intracerebrally with the challenge dose and its three dilutions, respectively. The challenge-dose control mice shall be injected last. The interval between the removal of the bacteria from the culture medium and the injection of the last mouse shall not exceed 2½ hours.

(d) *Recording the results.* The mice shall be observed for 14 days. Mice dying within 72 hours after challenge shall be excluded from the test. Records shall be maintained of the number of mice that die after 72 hours and of the number of mice showing both paralysis and enlargement of the head at the end of 14 days. All mice that show both paralysis and enlargement of the head shall be considered as deaths for the purposes of determining the ED_{50}.

(e) *Validity of the test.* The test shall be valid provided (1) the ED_{50} of the vaccine under test and the standard vaccine is between the largest and smallest vaccinating doses; (2) the limits of one standard deviation of each ED_{50} fall within the range of 64 percent to 156 percent; (3) the protective response is graded in relation to the vaccinating doses; (4) the dose-response curves of the vaccine under test and the standard vaccine are parallel; (5) the challenge dose contains approximately 200 LD_{50}; (6) the LD_{50} contains no more than 300 colony forming units; and (7) the $^1/_{1250}$ dilution of the challenge dose contains no less than 10 and no more than 50 colony forming units.

(f) *Estimate of the potency.* The ED_{50} of each vaccine shall be calculated by a